PATENT SPECIFICATION

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COMPLETE SPECIFICATION

NO DRAWINGS

Improvements in or relating to Particle Size Reduction or Cellular Disruption

We, BOOTS PURE DRUG COMPANY LIMITED, a British Company, of Station Street, Nottingham, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to an improved process for the production of solids in the form of extremely small particles and for the disruption of cells. It further relates to a novel apparatus in which said process may be carried out.

It is well-known that in many fields it is desirable to be able to produce powdered materials of an extremely small particle size. A typical example is in the field of pharmacy, wherein it may be necessary to provide suspensions of insoluble medicaments for injection in a form such that there is rapid and substantially complete absorption of the medicament by the body; for this it is essential that the medicament is in as finely-divided a state as possible. Ball milling and micronising are two techniques which are widely used for the production of powders of small particle size and using these techniques it is generally possible to produce powders of particle size ranging from 5 μ to 20 μ. It is very much easier to obtain powders of small particle size with relatively hard materials than it is with the relatively soft materials frequently encountered in pharmacy. It is also well-known that the efficient disruption of cellular material, for example biological cells such as bacteria, is a difficult problem.

It is an object of the present invention to provide an improved process for the production of powders of extremely small particle size which will work satisfactorily using soft materials and which will provide particles of a size much smaller than that possible using a ball mill or Microniser (Registered Trade Mark). It is a further object of the invention to provide a process for the disruption of cells which is more efficient than processes known hitherto. A still further object of the invention is the provision of an apparatus in which these processes can be carried out.

According to one aspect of the present invention there is provided a process for reducing the particle size of a particulate material which comprises subjecting to ultrasonic vibrations a suspension of said material in a chemically inert liquid in the presence of a plurality of small chemically inert bodies. The process may also be applied to the disruption of cellular material.

It is known that ultrasonic radiation alone at high power densities may be used for the disruption of cells. However by means of the process of the present invention it is possible to utilise ultrasonic radiation the energy of which is much lower than would be required for ultransonic radiation alone to be effective.

The chemically inert liquid in which the particulate or cellular material is suspended will generally be water but any liquid which does not dissolve or react with the material may be used. It may be convenient to include in the liquid a small amount of a surface active agent so that the material may be produced in the form of a well-dispersed suspension. The small chemically,inert bodies may be made from any substance which is chemically inert with respect to the particulate or cellular material, the suspending liquid and the surface active agent and which possesses the physical strength necessary to withstand the vibrations caused by the ultrasonic waves without deterioration. Glass beads or stainless steel balls are typical examples.

According to another aspect of the inven-

tion there is provided an apparatus in which particulate material may be reduced in particle size or cellular material may be disrupted which comprises in combination a vessel containing a plurality of small bodies, an ultrasonic transducer arranged such that the small bodies may be subject to ultrasonic vibration and means for bringing about additional agitation of the contents of the vessel.

The apparatus of the invention may be arranged in a number of ways. Thus for example there may be provided a vessel, to the base of which is attached a transducer, containing a layer of glass beads or like small inert bodies. Alternatively the beads may be placed in an inner container and the transducer attached to the base of an outer container, the intervening space being filled with a liquid such as water through which the ultrasonic radiation is transmitted. For cell disruption, the former arrangement has been found to give satisfactory results, but for particle size reduction the latter arrangement has been found to be preferable. A further alternative comprises a probe with attached ultrasonic transducer inserted into a vessel containing the beads. It will be appreciated that the vessel containing the beads or like bodies may be arranged with suitable inlets and outlets so as to allow operation on a continuous basis if desired.

For particle size reduction or cell disruption, sufficient of a suspension of the material to be treated in an inert liquid is added to the container holding the beads just to cover the beads and the transducer operated for a time sufficient to achieve the desired results.

The time taken to produce particles of an extremely small size or to disrupt cells is considerably reduced if the suspension of material to be treated and the small inert bodies are agitated by a means additional to the ultrasonic vibration. Thus for example the materials may be stirred. Therefore according to a preferred embodiment of the invention there is provided a process for reducing the particle size of a particulate material or disrupting cellular material which comprises subjecting to ultrasonic vibrations an agitated suspension of said material in an inert liquid in the presence of a plurality of small inert bodies, the agitation being brought about by a means additional to the ultrasonic vibration. The preferred apparatus of the invention includes a means for providing the additional agitation, such as for example stirring means. A hollow stirrer may be employed so as to function as an inlet for operation of the apparatus on a continuous basis.

It will be appreciated that the values of the many variables which occur in the process of the invention cannot be stated in specific terms to cover all eventualities. These variables include the power input, the size of the inert bodies, the depth of the layer of the inert bodies, the dimensions of the containers, the concentration of the suspension etc. and they must be determined by experiment for individual cases. In particular the conditions for optimum efficiency will clearly vary from application to application.

We have found that satisfactory results are obtained using a combination of a transducer with a power output of about 30 watts and a frequency of 20-40 kilocycles, glass beads 0.2-2 mm. in diameter and at a depth of about 1 inch in a vessel about 3 inches in diameter. Both size and depth of beads may be increased if a more powerful transducer is used. For the best results it has been found that the relationship between bead size and the initial particle or cell size is an important factor. Thus for example, glass beads 0.2 mm. in diameter have been shown to be superior to 1 mm. diameter beads for the disruption of *Staphylococci* about 0.5 μ in diameter. On the other hand for the disintegration of yeast cells about 3 μ in diameter, the larger beads have been found to give superior results. The initial particle or cell size should preferably be considerably smaller than the bead size. For example a suspension of material 0.1 mm. in diameter is effectively reduced in particle size using 1 mm. beads.

In the process of the invention carried out in a double container as hereinbefore described, the diameter of inner and outer containers should be similar for maximum efficiency. The distance between the bases of the containers (the outer one having a transducer affixed to it) appears to have an optimum value corresponding to half the wavelength of the ultrasonic radiation in the liquid used to fill the intervening space, e.g. about 1 inch for water using 20-40 kilocycle radiation.

As stated hereinbefore, the inert liquid in which the material to be treated is suspended will frequently be water as it is often desirable to obtain an aqueous suspension of a finely divided solid, for example a medicament. However, if it is desired to obtain a dry powder of extremely small particle size, it may be advantageous to employ a volatile substantially anhydrous liquid, such as benzene or ether, as suspending liquid. In this case, after carrying out the process of the invention, the fine suspension is isolated and the liquid removed for example by spray drying. Another useful procedure may be to use as the suspending liquid a solution of an oinntment base, such as petroleum jelly, in a volatile liquid. In this case, after the process of the invention has been completed the volatile solvent is easily removed to leave

a highly dispersed suspension of the fine particles in the ointment base.

The following non-limitative examples illustrate the invention.

Example 1

A glass beaker, 3 inches in diameter, containing a 1½ inch layer of 1 mm. glass beads and fitted with a stirrer, was arranged inside an outer vessel containing water, to the base of which was attached a transducer. A 40 kilocycle generator with about 100 watts ultrasonic power output was employed. A suspension of micronised procaine penicillin (4 grams, in 20 ml. of ether) was added to the beaker, stirred slowly, and ultrasonic radiation applied for 40 minutes.

Initially the micronised material comprised 5-10 μ particles; the final particle size was 1 μ.

Example 2

In the apparatus described in Example 1, 2 grams of a steroid in sufficient water to cover the beads was treated for 30 minutes. The initial particle size was 100 μ; at the end of the 30 minute treatment, no particles were greater than 3 μ. As a further indication of the particle size reduction achieved, the initial sedimentation volume was 8ml. the final 120ml.

Example 3

In an apparatus similar to that described in Example 1 but containing a ¾ inch layer of 0.2 mm. glass beads, a concentrated suspension of *Staphylococcus aureus* in sufficient water to cover the beads was treated. Cell disruption was estimated by cell wall count by electron microscopy. After 5 minutes treatment 20% cell disruption was achieved and after 45 minutes, 100% cell disruption was observed.

Example 4

The apparatus used in this example comprised a 4 inch diameter vessel attached to the base of which was a transducer, with a 20 kilocycle generator, about 35 watts output. The vessel was fitted with a stirrer and contained a ¾ inch layer of 1 mm. beads. Fresh yest (4 grams as a 25% aqueous dispersion) was added to the vessel and subjected to ultrasonic vibrations with stirring. Temperature measurements indicated that the power utilised was about 10 watts. The discrepancy between this figure and the nominal output of the transducer was due to the lack of accurate matching of the transducer to the load. After 2 minutes, 52% cell disruption had been achieved and, after 6 minutes 75% cell disruption (estimated by cell wall counts in stained smears).

A similar experiment carried out in the apparatus, containing no glass beads showed inappreciable cell disruption after 12 minutes.

Example 5

A glass beaker, 2 inches in diameter, containing an aqueous paste of 1 gram of sulphur and 0.1% sodium carboxymethylcellulose, and a half inch layer of 1 mm. glass beads was placed in a bath of water, to which ultransonic vibrations were transmitted from a transducer. The contents of the beaker were stirred slowly during this time. Initially the particle size distribution curve of the sulphur showed a peak at 100 μ. After carrying out the process of the invention for 40 minutes the maximum particle size of the sulphur was 2 μ.

WHAT WE CLAIM IS:—

1. A process for reducing the particle size of a particulate material which comprises subjecting to ultrasonic vibrations a suspension of said material in a chemically inert liquid in the presence of a plurality of small chemically inert bodies.

2. A process as claimed in claim 1, which comprises subjecting to ultrasonic vibrations an agitated suspension of said material in a chemically inert liquid in the presence of a plurality of small chemically inert bodies, the agitation being brought about by a means additional to the ultrasonic vibration.

3. A process as claimed in claim 2, in which stirring is used to provide the agitation.

4. A process as claimed in any of claims 1 to 3, in which the small chemically inert bodies comprise glass beads or stainless steel balls.

5. A process as claimed in any of claims ~~1 to 4, in~~ which the chemically inert liquid is water.

6. A process as claimed in any of claims 1 to 4. in which the chemically inert liquid is a volatile substantially anhydrous liquid.

7. A process as claimed in any of claims 1 to 6, in which the liquid contains a surface active agent.

8. A modification of the process as claimed in any of claims 1 to 7 in which, instead of particulate material being reduced in particle size, cellular material is disrupted.

9. A process as claimed in any of claims 1 to 7, and substantially as herein described particularly with reference to Examples 1, 2 and 5.

10. A process as claimed in claim 8 and substantially as herein described particularly with reference to Examples 3 and 4.

11. Apparatus in which particulate material may be reduced in particle size or cellular material may be disrupted which comprises in combination a vessel containing a plurality of small bodies, an ultrasonic transducer arranged such that the small bodies may be subject to ultrasonic vibration, and a means for bringing about additional agitation of the contents of the vessel.

12. An apparatus as claimed in claim 11, in which said means for bringing about additional agitation comprises a stirrer.

13. An apparatus as claimed in claim 11 or claim 12, in which the small bodies comprise glass beads or stainless steel balls.

14. An apparatus as claimed in any of claims 11 to 13 in which the transducer is attached to the base of the vessel containing the plurality of small bodies.

15. An apparatus as claimed in any of claims 11 to 13 comprising an inner vessel containing the small bodies and an outer vessel to which is attached a transducer.

16. An apparatus as claimed in claim 15, in which the intervening space between said inner and outer vessel contains a liquid.

17. An apparatus as claimed in any of claims 11 to 16 and adapted for use on a continuous basis by the provision of suitable inlet and outlet means.

18. An apparatus as claimed in claim 12, in which the stirrer is hollow and serves as an inlet for operation of the apparatus on a continuous basis.

19. An apparatus as claimed in any of claims 11 to 18 and substantially as herein described, particularly with reference to the Examples.

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